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EFFECTS OF DIFFERENT CALCIUM CONCENTRATIONS SUPPLEMENTED ON THE DIET OF *PARTULA GIBBA* ON THEIR MORPHOMETRIC GROWTH PARAMETERS, WEIGHT AND REPRODUCTION SUCCESS

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ABSTRACT

Partula gibba, commonly known as the Fat Guam *Partula*, is endemic to Guam and Northern Mariana Islands, and it is listed as endangered in the IUCN Red List of Threatened Species. The effects of calcium concentration on its growth and reproductive success were studied.

The snails were divided into six groups, fed *ad libitum* on a diet based on the one formulated by Frame and Clarke at the Nottingham University approximately 25 years ago or variants in which cuttlebone was substituted by various concentrations of calcium carbonate. Shell height, thickness and width, aperture/lip height and width where recorded every month for a whole year, and all were found to be significantly positively correlated with the percentage of added calcium carbonate in the diet (range 0–40% dry wt:wt). Morphometric growth parameters of snails fed 20% added calcium carbonate did not differ significantly from the diet with added cuttlebone (20%), with the exception of their reproductive success, which was lower with the cuttlebone treatment. Reproduction success (expressed by the number of newborns per adult per month) was also significantly and positively correlated over the range of added calcium carbonate to the diet.

Key words: *Partula* snails, calcium, growth parameters, reproductive success, diet.

INTRODUCTION

Partula gibba, commonly known as the Fat Guam *Partula*, is endemic to Guam and the Northern Mariana Islands, and is listed as endangered in the IUCN Red List of Threatened Species.

Gastropod distributions may depend on various factors, but calcium concentration has been found to be a particularly important and limiting (Brodersen & Madsen, 2003). The importance of calcium to terrestrial gastropods is well known, and the observations are almost always similar to the early description of Boycott (1934), who showed that dietary calcium is essential for growth, reproduction and shell construction (Wagge, 1952; Crowell, 1973; Ireland, 1991; Ireland & Marigomez, 1992; Egonmwan, 2008). The snails' shell comprises approximately 97% calcium carbonate and this accounts for their highly demand for this element (Heller & Magaritz, 1983).

Several studies have examined the effects of calcium concentration on various snail species (Brodersen & Madsen, 2003; Harrison, 1970; Madsen, 1987), and these have mostly concluded that the snails are generally able to live under low calcium concentrations in the laboratory, but that growth is inhibited and thinner shells are usually developed (Brodersen & Madsen, 2003). Crowell (1973) demonstrated that plant matter alone is not a sufficient source of calcium, and observed that the shells of snails fed only on plant matter were weak, thin and fragile. He also showed experimentally that *Helix aspersa* ingested soil, and that the soil subsequently passed as faeces contained 12–32% less calcium than soil with no previous exposure to snails (Heller & Magaritz, 1983), and therefore soil is likely to be an important source of calcium in the wild.

The diet of *Partula* snails in the wild is largely unknown. They are detritivores, but have been observed feeding on various plants includ-

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ing decaying stalks of *Hibiscus* (Malvaceae), *Etlingera* (Zingiberaceae), *Piper* (Piperaceae), *Microsorum* (Polypodiaceae), and *Cocos* (Arecaceae) (Hopper & Smith, 1992; Cunningham et al.,1996; Coote, 2007).

Partula are ovoviviparous hermaphrodites (Clarke & Murray, 1969), and they produce a completely shelled offspring every six to eight weeks.

In order to test whether calcium plays an important role in the wellbeing of the *Partula* snail cultures, we investigated the effect of different dietary mixes varying in their calcium carbonate content.

METHODS

The snails *Partula gibba* used in the experiment were the offspring from the parents from London Zoo population. Individuals were maintained in a controlled temperature room (range of 22°C at night and 23°C during the day). Relative humidity varied between 70 and 80%. Light was provided by fluorescent tubes on a twelve hour day and night cycle.

Because of the high mortality experienced during the first post natal week, *Partula gibba* were used after they reached a minimum weight of 0.03 grams and a maximum of 0.05 grams, the snails were then randomly placed into individual plastic containers (250 ml) covered with cling film®, the substratum consisted of layers of damp bleach-free paper tissue.

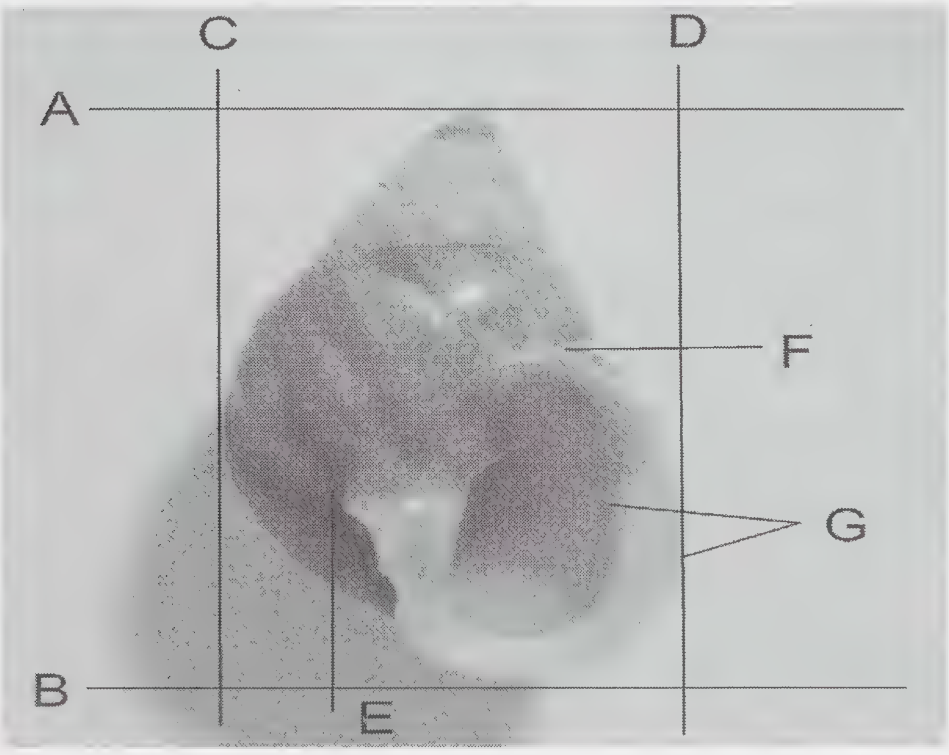

FIG. 1. Snails' morphometric measurements; shell thickness (G), height (A–B), width (C–D), lip height (B–F) and lip width (E–D).

Snails were fed *ad libitum* on a diet based on the one formulated by Frame and Clarke at the Nottingham University approximately 25 years ago (Tonge & Bloxam, 1990), 300 g of porridge oats, 150 g of powdered trout pellets, 600 g powdered grass pellets, 25 g of vitamin powder (Vetzyme, Seven Seas Ltd.) and 300 g of powdered cuttlebone. The diet was moistened with filtered water to form a runny paste and then microwaved for approximately two minutes for sterilisation.

Different groups of snails were fed either standard diet or variants in which the cuttlebone was replaced by powdered calcium carbonate 0, 10, 20, 30 and 40% (wt:wt)(Mineral Technologies, Inc.). There were 10 replicates of each treatment. The diet, tissue and the containers were replaced every month, and the numbers of newborns (reproduction success) were counted. All the materials used to handle, feed, house and clean the snails were previously sterilised/disinfected in a microwave or with water sterilized with Milton tablets (sodium hypochlorite) (Coates, 1996).

Snails were weighed once per month from the beginning of the experiment until the end. Due to their thin delicate shells, morphometric measurements were only conducted once the snails reached the adults stage (characterized by the formation of the lip (P. Pearce-Kelly, personal observation)). Shell height, thickness and width, aperture/lip height and width of each snail were measured using a digital vernier calliper, based on the pathology protocol in use at the Zoological Society of London, Pathology Department (Fig. 1). The snails' measurements were recorded once per month for a whole year.

Statistical analyses of the data were performed using the software package R (Ihaka & Gentleman, 1996).

RESULTS

Results for changes in morphometric parameter between each monthly measurement are showed in Figs. 2–8. All morphological measures as well as snail weight were found to be significantly and positively correlated with the percentage of added calcium carbonate in the diet (Table 1.).

In addition, reproductive success (expressed by the number of newborns per adult per month) was significantly and positively correlated over the range of added calcium carbon-

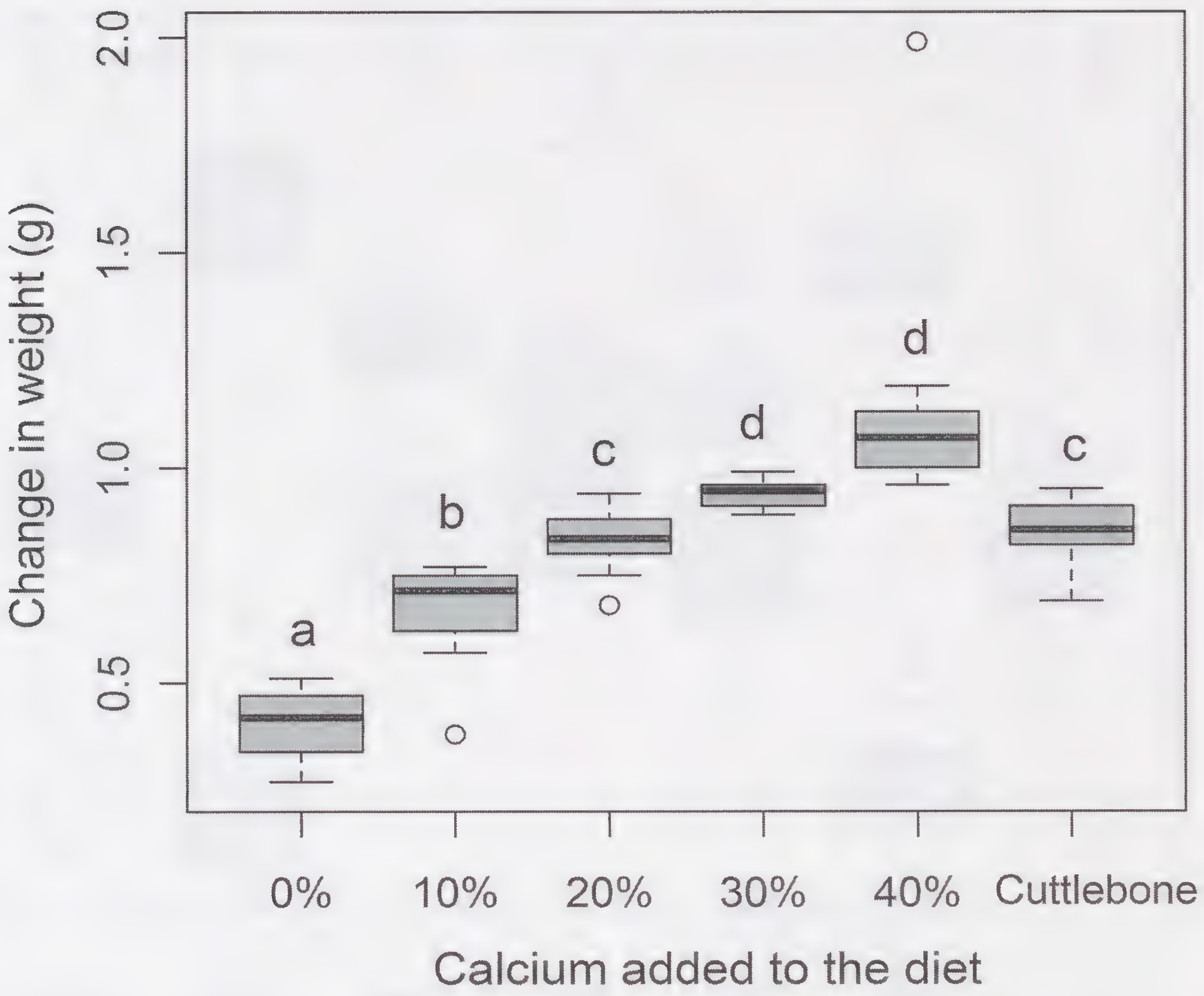

FIG. 2. Effects of different calcium carbonate concentrations added to the diet including 20% powdered cuttlebone on the weight of the snails. The top and bottom of each rectangular box denote the 75th and 25th percentiles, respectively, with the median shown inside the box. Horizontal bars on lines extending from each box represent the 90th and 10th percentiles, and the outliers are illustrated as "○". Significant differences among individual treatments are denoted with letters over each box. (Statistics are presented in the same format for all of the box plots in this study).

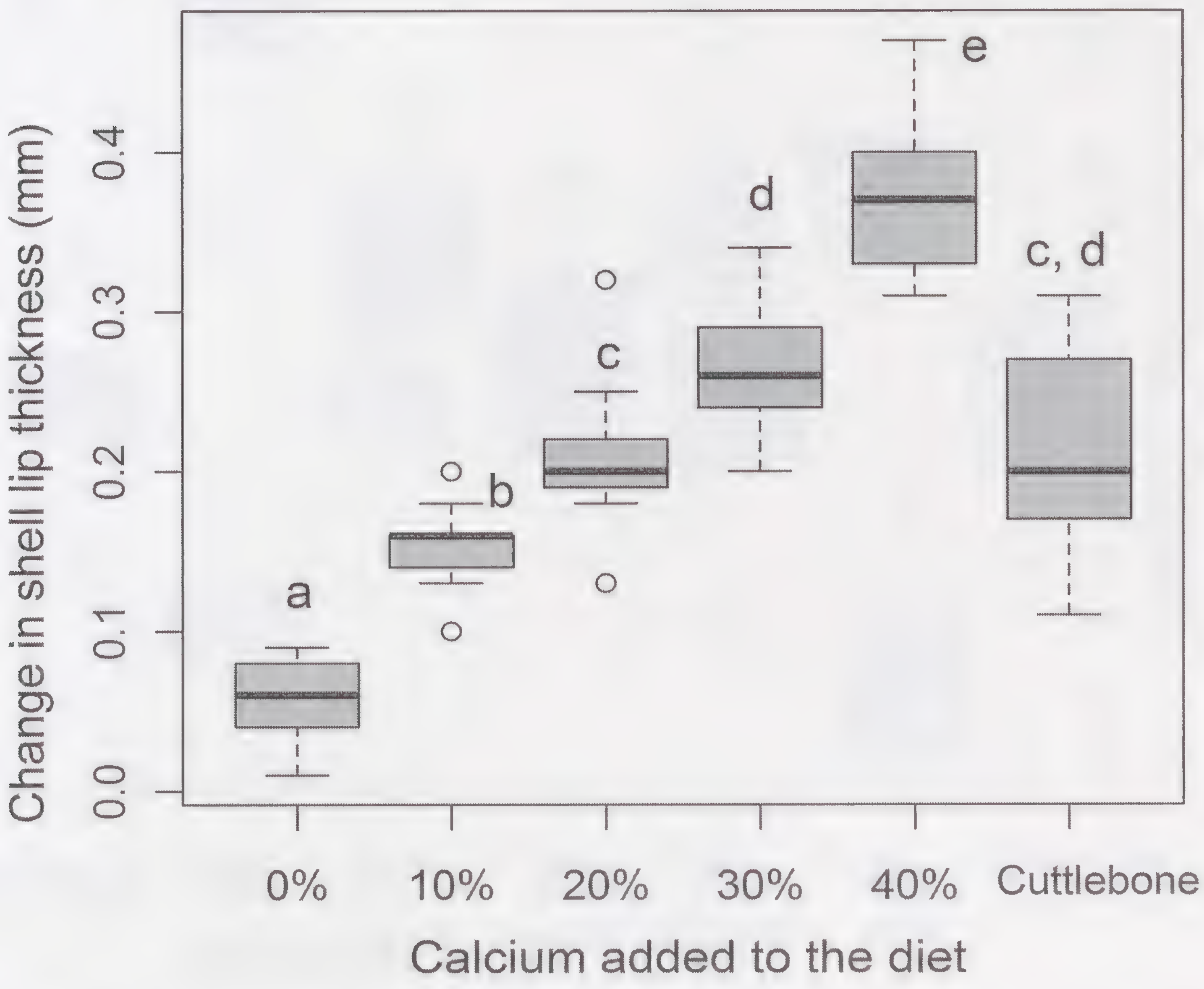

FIG. 3. Effects of different calcium carbonate concentrations added to the diet including powdered cuttlebone on the snails' shell lip thickness.

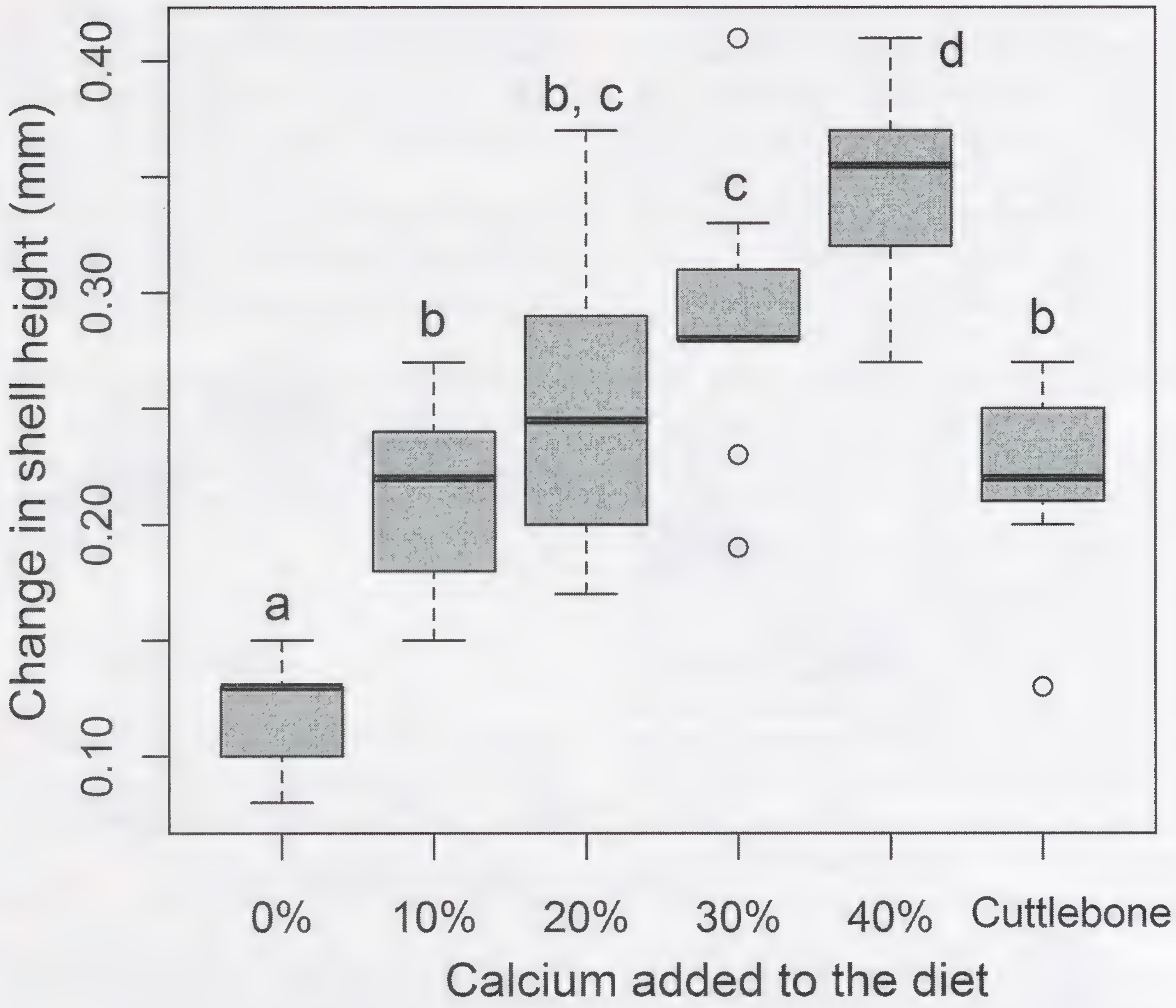

FIG. 4. Effects of different calcium carbonate concentrations added to the diet including powdered cuttlebone on the snails' shell height.

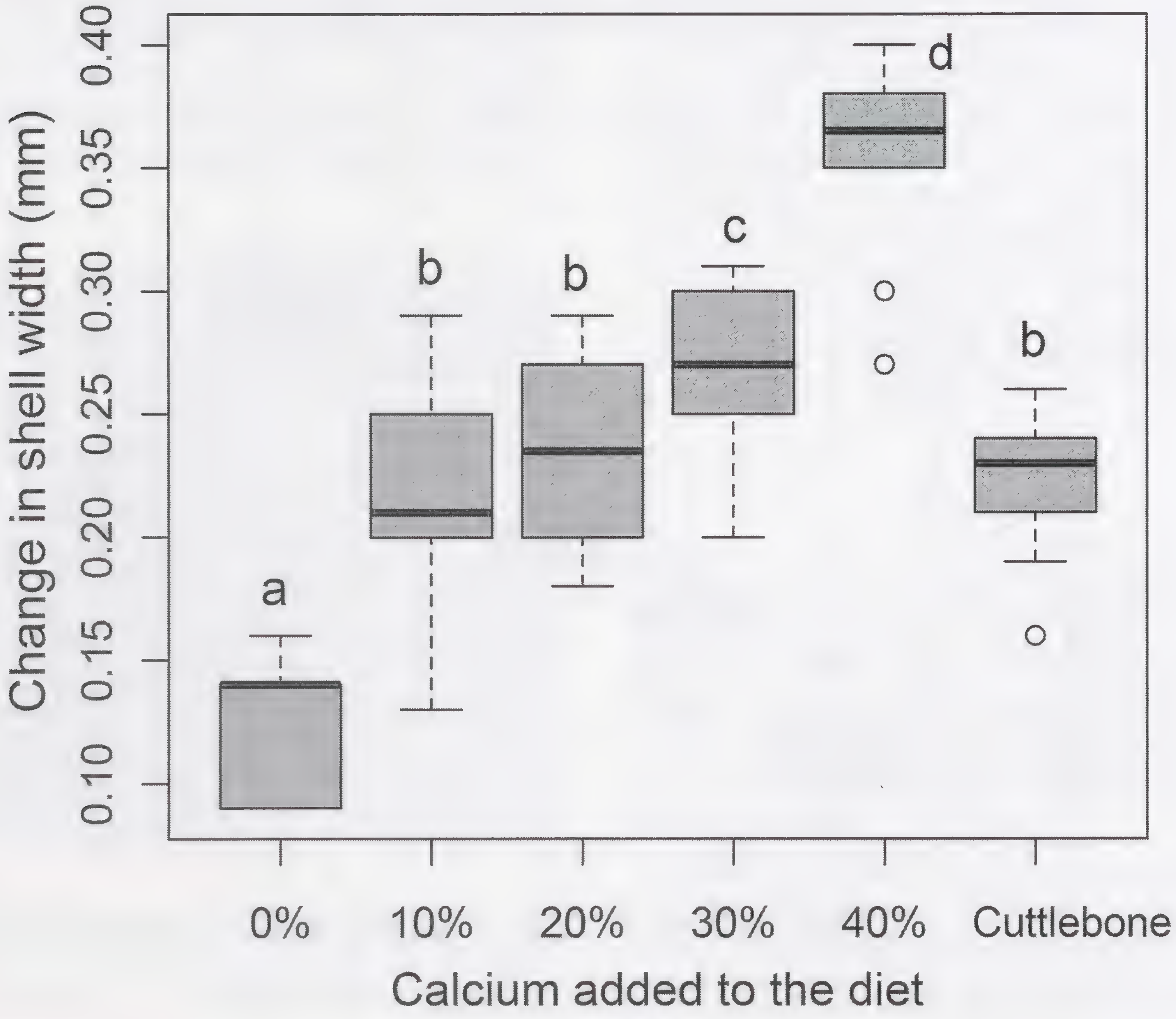

FIG. 5. Effects of different calcium carbonate concentrations added to the diet including powdered cuttlebone on the snails' shell width.

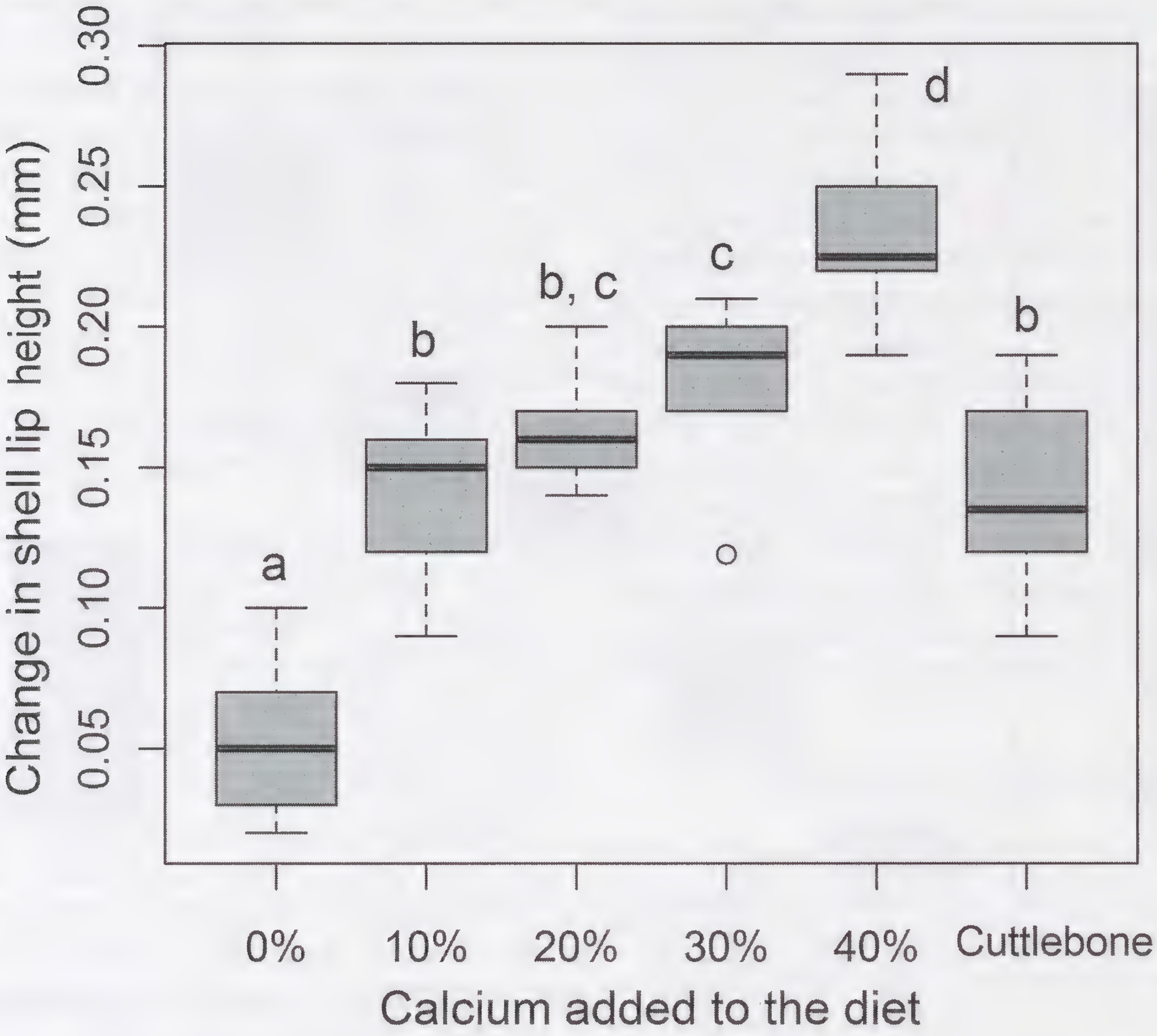

FIG. 6. Effects of different calcium carbonate concentrations added to the diet including powdered cuttlebone on the snails' shell lip height.

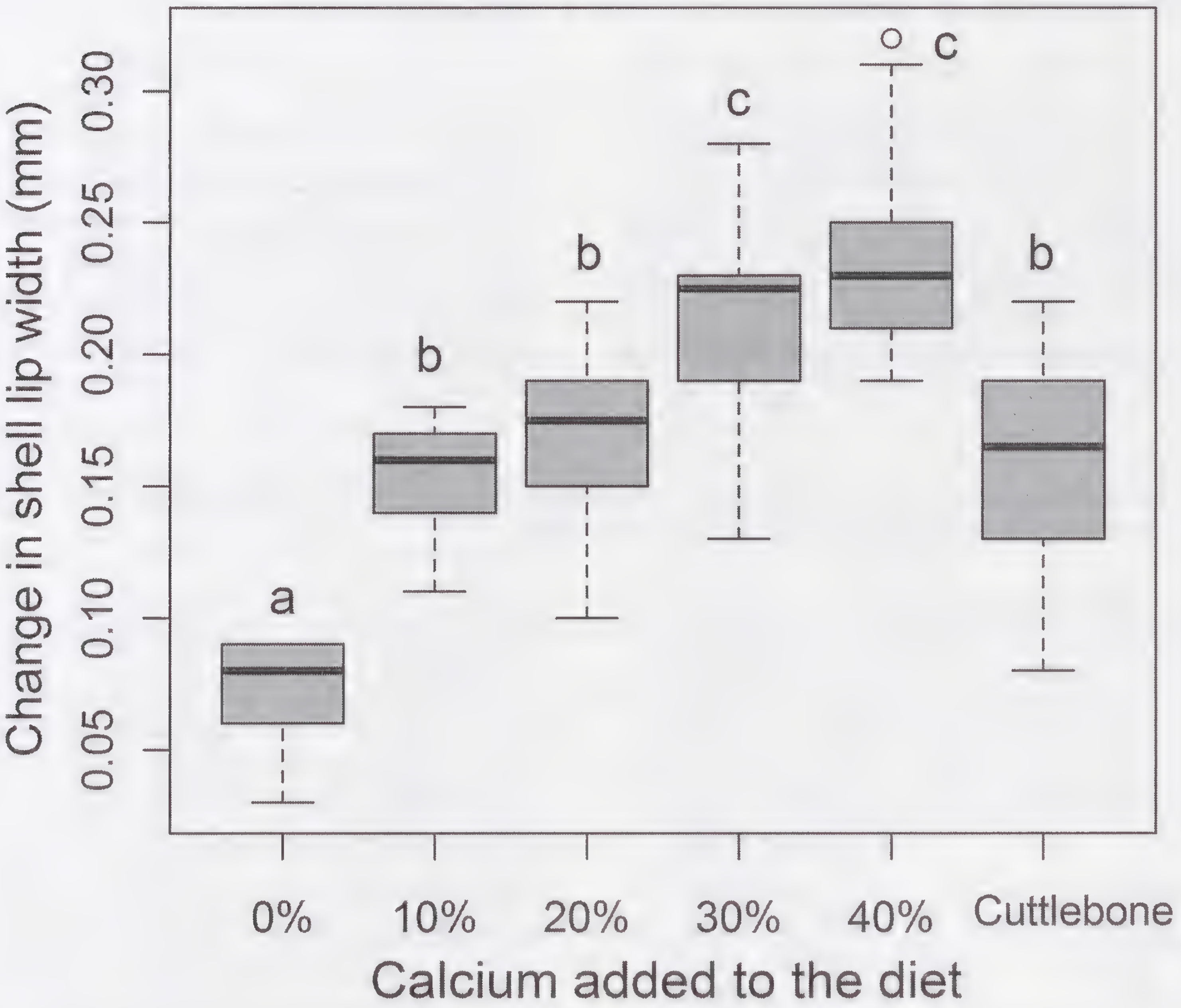

FIG. 7. Effects of different calcium carbonate concentrations added to the diet including powdered cuttlebone on the snails' shell lip width.

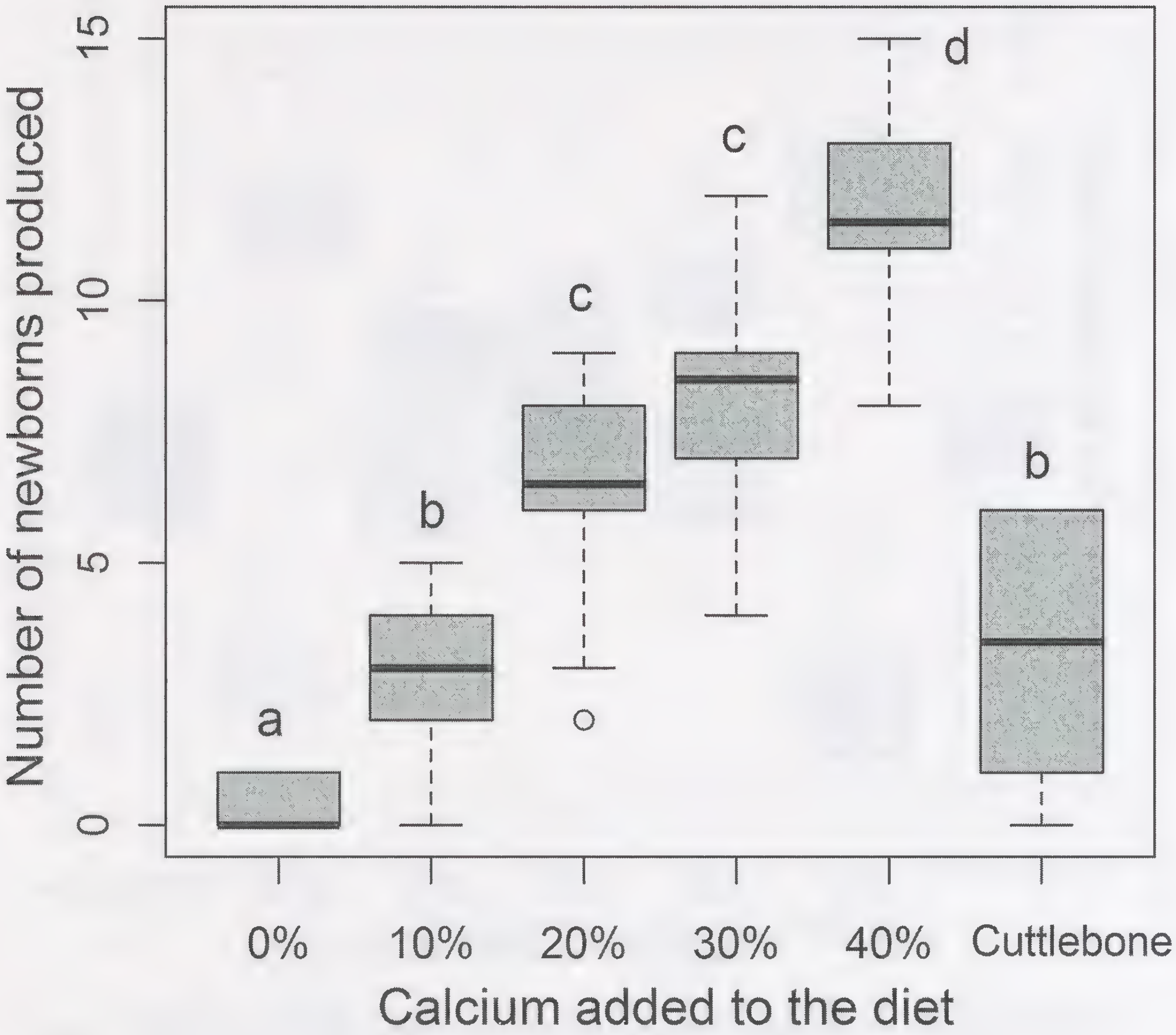

FIG. 8. Effects of different calcium carbonate concentrations added to the diet including powdered cuttlebone on the reproductive success of *Partula gibba,* expressed as number of newborns per month over a year period.

ate to the diet. Nearly all parameters showed an approximately linear relationship.

Visual inspection of the graphs and t-tests showed that the morphometric growth parameters of snails fed 20% added calcium carbonate did not differ significantly from the diet with added cuttlebone (20%) with the exception of their reproductive success where there is a significant difference ($t = 2.9$, $df = 17.986$, $p < 0.01$), the diet with added 20% calcium carbonate has a higher reproduction success than the diet with cuttlebone (20%).

TABLE 1. Linear model summary, for *Partula gibba* morphometric measures and reproduction success against the addition of calcium to the diet.

	F-stats	p-value	r^2	Intercept
Snails' weight	$F_{1,48} = 131.3$	$P < 0.001$	0.73	$P < 0.001$
Shell lip thickness	$F_{1,42} = 215.8$	$P < 0.001$	0.84	$P < 0.001$
Shell height	$F_{1,42} = 75.72$	$P < 0.001$	0.64	$P < 0.001$
Shell width	$F_{1,42} = 108.8$	$P < 0.001$	0.72	$P < 0.001$
Shell lip height	$F_{1,42} = 120.8$	$P < 0.001$	0.74	$P < 0.01$
Shell lip width	$F_{1,42} = 77.45$	$P < 0.001$	0.65	$P < 0.05$
Reproductive success	$F_{1,48} = 223.6$	$P < 0.001$	0.82	ns

DISCUSSION

Nutrition is one of the major factors that affects the performance of livestock (Oluokun et al., 2005). Following this line of thought, this study focused on the importance of calcium in the development and reproduction success of *Partula gibba*. The results presented in this study are consistent with the study performed by Oluokun et al. (2005), which concluded that the snails' shell height, width and thickness increased with an increased calcium concentration in the diet.

Partula snails are nutrient recyclers and hence have an important role in their ecosystem. The total disappearance of *Partula* snails in the wild can be devastating to the ecosystem and further studies on the effect of their extinction upon their ecosystem should be considered. For example, Gärdenfors et al. (1995) noted that the abundance and diversity of snails are closely positively correlated to pH in the soil (Johannessen & Solhøy, 2001). Johannessen & Solhøy (2001) concluded that snail populations have a positive effect upon bird population since some species rely on the snails shell as a source of calcium. These studies indicate the importance of snails as a source of calcium in forest ecosystems.

As *Partula* snails face an uncertain future with the high rate of species extinction currently observed, it is of great importance that all the factors contributing to their welfare are fully understood. This study demonstrated that an increased level of dietary calcium carbonate significantly improved their wellbeing.

Numerous studies previously demonstrated the importance of calcium as a factor determining the development and wellbeing of snails. Fournie & Chetail (1982), for example, established that the addition of calcium carbonate to the snails' diet prevented them from dwarfing, with the exception of snails reared on calcareous soils (Elmslie, 1988; Egonmwan, 2008). Calcium also plays a vital role on the regeneration process of the snails' shell. Bierbauer (1957) reported that shell repair in *Helix pomatia* was enhanced following injection of calcium (Vaidya, 1980). Voelker (1959) noted that scarcity of calcium may result in thinner and more brittle shells, rendering them less fit to protect the snail properly against desiccation, physical damage and invertebrate predators (Johannessen & Solhøy, 2001). Egonmwan (2008) concluded that calcium deficiency in *Limicolaria flammea* resulted in a decrease in the growth rate of these snails raised in the laboratory. All these studies demonstrate that calcium is an important nutrient in the snails' life.

It is therefore recommended that an increase of calcium concentration to 40% instead of 20%, in the standard diet of *Partula* snails would be beneficial and that calcium carbonate powder should be used instead of cuttlebone, since the cuttlebone diet did not demonstrate any additional benefits. It is also known that the cuttlebone can be contaminated with heavy metals (Miramand et al., 2006), such as mercury (Hg) (Lacoue-Labarthe et al., 2009), bioaccumulation of PCBs (Danis et al., 2005) and potentially infectious agents, hence calcium carbonate is effortless to use and it is simpler to control possible routes of contamination that may influence the snails' welfare.

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LITERATURE CITED

BIERBAUER, J., 1957, Untersuchungen über die regeneration und histologie von *Helix pomatia*. *Acta Biologica Academiae Scientiarum Hungaricae*, 7: 419–431.

BOYCOTT, A. E., 1934, The habitats of land mollusca in Britain. *Journal of Ecology*, 22: 1–38.

BRODERSEN, J. & H. MADSEN, 2003, The effect of calcium concentration on the crushing resistance, weight and size of *Biomphalaria sudanica* (Gastropoda: Planorbidae). *Hydrobiologia*, 490: 181–186.

CLARKE, B. & J. MURRAY, 1969, Ecological genetics and speciation in land snails of the genus *Partula*. *Biological Journal of the Linnean Society*, 1: 31–42.

COATES, D., 1996, Sporicidal activity of sodium dichloroisocyanurate, peroxygen and glutaraldehyde disinfectants against *Bacillus subtilis*. *Journal of Hospital Infection*, 32: 283–294.

COOTE, T., 2007, Partulids on Tahiti: differential persistence of a minority of endemic taxa among relict populations. *American Malacological Bulletin*, 22: 83–87.

CROWELL, H. H., 1973, Laboratory study of calcium requirements of the brown garden snail, *Helix aspersa* Muller. *Journal of Molluscan Studies*, 40: 491–503

CUNNINGHAM, A. A., P. DASZAK, S. K. MACGREGOR, I. FOSTER, D. CLARKE & P. PEARCE-KELLY, 1996, Mortality of endangered snails of the genus *Partula*: preliminary results of pathologic investigations. *Journal of Zoo and Wildlife Medicine*, 27: 19–27.

DANIS, B., P. BUSTAMANTE, O. COTRET, J. L. TEYSSIÉ, S. W. FOWLER & M. WARNAU, 2005, Bioaccumulation of PCBs in the cuttlefish *Sepia officinalis* from seawater, sediment and food pathways. *Environmental Pollution*, 134: 113–122.

EGONMWAN, R. I., 2008, Effects of dietary calcium on growth and oviposition of the African land snail *Limicolaria flammea* (Pulmonata: Achatinidae). *Revista de Biologia Tropical*, 56: 333–343.

ELMSLIE, L. J., 1988, Studies on the feeding of newly hatched *Helix aspersa*. *Snail Farming Research*, 2: 45–48.

FOURNIE, J. & M. CHETAIL, 1982, Evidence for a mobilization of calcium reserves for reproduction requirements in *Deroceras reticulatum* (Syn: *Agriolimax reticulatus*) (Gastropoda: Pulmonata). *Malacologia*, 22: 285–291.

GÄRDENFORS, U., H. W. WALDÉN & I. WÄREBORN, 1995, Effects of soil acidification on forest land snails. *Ecological Bulletin*, 44: 259–270.

HARRISON, A. D., 1970, Studies on the effects of calcium bicarbonate concentrations on the biology of *Biomphalaria pfeifferi* (Krauss) (Gastropoda: Pulmonata). *Hydrobiologia*, 36: 317.

HELLER, J. & M. MAGARITZ, 1983, From where do land snails obtain the chemicals to build their shells? *Journal of Molluscan Studies*, 49: 116–121.

HOPPER, D. R. & B. D. SMITH, 1992, Status of tree snails (Gastropoda: Partulidae) on Guam, with a resurvey of sites studied by H. E. Crampton in 1920. *Pacific Science*, 46: 77–85.

IHAKA, R. & R. GENTLEMAN, 1996, R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, 5: 299–314.

IRELAND, M. P., 1991, The effect of dietary calcium on growth, shell thickness and tissue calcium distribution in the snail *Achatina fulica*. *Comparative Biochemistry and Physiology* (*Part A: Physiology*), 98: 111–116.

IRELAND, M. P. & I. MARIGOMEZ, 1992, The Influences of dietary calcium on the tissue distribution of Cu, Zn, Mg & P and histological changes in the digestive gland of the snail *Achatina fulica* Bowdich. *Journal of Molluscan Studies*, 58: 157–168.

JOHANNESSEN, L. E. & T. SOLHØY, 2001, Effects of experimentally increased calcium levels in the litter on terrestrial snail populations. *Pedobiologia*, 45: 234–242.

LACOUE-LABARTHE, T., M. WARNAU, F. OBERHÄNSLI, J.-L. TEYSSIÉ & P. BUSTAMANTE, 2009, Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis* exposed to 203Hg radio labelled seawater and food. *Aquatic Biology*, 6: 91–98.

MADSEN, H., 1987, Effect of calcium concentration on growth and egg laying of *Helisoma duryi, Biomphalaria alexandrina, B. camerunensis* and *Bulinus truncatus* (Gastropoda: Planorbidae). *Journal of Applied Ecology*, 24: 823–836.

MIRAMAND, P., P. BUSTAMANTE, D. BENTLEY & N. KOUETA, 2006, Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis. Science of the Total Environment*, 361: 132–143.

OLUOKUN, J. A., A. J. OMOLE & O. FAPOUNDA, 2005, Effect of increasing the level of calcium supplementation in the diets of growing snail on performance characteristics. *Research Journal of Agriculture and Biological Sciences*, 1: 76–79.

TONGE, S. & Q. BLOXAM, 1990, A review of the captive-breeding programme for Polynesian tree snails *Partula* species. *International Zoo Yearbook*, 30: 51–59.

VAIDYA, D. P., 1980, Calcium regulation in the freshwater snail *Indoplanorbis exustus* during shell repair. *Hydrobiologia*, 69: 29–32.

VOELKER, J., 1959, Der chemische einfluss von kalziumkarbonat auf wachstum, entwicklung und gehäusebau von *Achatina fulica* Bowdich (Pulmonata). *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut der Universität Hamburg*, 57: 37–78.

WAGGE, L. E., 1952, Quantitative studies of calcium metabolism in *Helix aspersa*. *Journal of Experimental Zoology*, 120: 311–342.

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